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# GENETICS OF PATHOGENICITY IN MELAMPSORA LINI[1]

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## INTRODUCTION

Flax rust (*Melampsora lini* (Pers.) Lév.) occurs in the major flax-growing regions of the world and occasionally is very destructive. It was prevalent in Texas, Minnesota, and North Dakota in 1941; in the last-named State it caused total losses in certain fields and an average reduction in yield of about 20 percent, or more than 1 million bushels of flaxseed. In 1942 rust reduced the yield of flaxseed in North Dakota by 25 percent, or about 2 million bushels; but in 1943, although there was an increase in production of 40 percent, loss attributable to rust was about half a million bushels. The use of rust-resistant varieties was largely responsible for this reduction in loss from rust in 1943.

*Melampsora lini* is a long-cycle rust which produces all of its spore stages on the flax (*Linum usitatissimum* L.) plant. It comprises numerous physiologic races that differ sharply in their pathogenicity on certain flax varieties. Every one of more than 300 varieties obtained from the major flax-producing regions of the world is susceptible to one or more of these races, but no race attacks all varieties. The varieties which have been extensively used as rust-immune parents in an attempt to develop rust-resistant varieties in North America are highly susceptible to some races occurring in South America. Also, certain North American races attack the varieties that have been immune from rust in South America (*3*, *10*).[2] The United States imports large quantities of flaxseed from South America. Although practically all of this imported flaxseed is processed, occasionally some of it is used for seed. Since flax rust is commonly spread from one locality to another by means of telia on stem segments mixed with seed from rusted fields, it is possible that races virulent on varieties now regarded as resistant may become established. In the northern part of the United States *M. lini* overwinters in the telial stage only; thus, hybridization of this fungus probably is coincident with the establishment of infection each year. A study of the inheritance of pathogenicity in hybrids between North American and South American races of *M. lini* should yield information on the potential patho-

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[2] Italic numbers in parentheses refer to Literature Cited, p. 357.



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genicity of such hybrids and might facilitate the selection of resistant parental varieties.

Johnson and Newton (*7*) found that certain pathogenic characters of the fungus causing black stem rust of wheat (*Puccinia graminis tritici* Eriks. and E. Henn.) were inherited on a Mendelian basis. Flor (*5*) reported that avirulence was dominant in the $F_1$ generation of crosses between several North American races and between the North American and South American races of *Melampsora lini* studied in these investigations. Segregation in the $F_2$ generation of a cross of North American race 6 with race 24 was explained by assuming that single pairs of recessive factors conditioned virulence on Akmolinsk and Bombay, each of which possessed a single pair of rust-conditioning factors, and that two pairs of quantitative factors conditioned virulence on Buda, a variety having two pairs of factors for resistance to race 6 but moderately susceptible to race 24. This paper reports a continuation of these investigations.

The objectives of the present investigations were to determine: (1) What the potential variation is and how many physiologic races are possible; (2) whether allelomorphic or linkage relations exist between certain factors for pathogenicity in the pathogen comparable with those that exist between the factors that condition rust reaction in the host (*4*, *6*, *8*); and (3) whether the virulence of certain South American races on numerous varieties of flax of diverse type and origin, some of which are known to possess different factors for rust reaction, is due to a single pathogenic factor or to a number of them.

## MATERIAL AND METHODS

The parent races used in these studies, race 22 of South American origin and races 6 and 24 of North American origin, were differentiated by the reaction of 11 varieties of flax (*3*). Each of the rust-differentiating varieties was pure for reaction to the parent races, but approximately 25 percent of the plants of Argentine selection (C. I.[3] 462) were susceptible to some of the $F_2$ cultures used in the present studies. The plants susceptible to these $F_2$ cultures were immune from the other $F_2$ cultures for which this Argentine selection was pure for immunity. This indicated that duplicate factors conditioned susceptibility to race 22 and immunity from races 6 and 24 and that this Argentine selection was homozygous for at least 1 factor pair and heterozygous for 1 or more others.

These results also indicated that more than 1 factor pair was involved in conditioning virulence of race 22 on Argentine selection (C. I. 462) and suggested that for each factor for resistance in the host there was a specific factor for virulence in the pathogen. If this is true, varieties of the diverse group that are immune from races of North American origin and susceptible to races of South American origin (*3*) might have factors for rust reaction not possessed by any of the 11 differential varieties. A test of 16 varieties that had been included in this group (*3*) with several $F_2$ cultures showed that at least 5 had differential reactions distinct from those of the 11 differential varieties then in use and that a sixth, Morye (C. I. 112), a

[3] C. I. refers to accession number of the Division of Cereal Crops and Diseases.

selection made by Burnham (*1*), apparently was homozygous for the 3 pairs of rust-conditioning factors possessed by Argentine selection (C. I. 462). Consequently, the 5 varieties Newland (C. I. 188), Bolley Golden [4] (C. I. 644), Italia Roma (C. I. 1005–2), Leona (Argentine hybrid, C. I. 836), and Tammes Pale Blue (C. I. 333–1) have been added to the rust-differentiating varieties already in use (*3*), and Morye has replaced Argentine selection (C. I. 462), making a total of 16 differentials.

The investigations reported here cover pathogenicity tests of the $F_2$ cultures of crosses of race 22 of South American origin with races 6 and 24 from North America. All of the differential varieties except J. W. S. and Bombay are highly susceptible to race 22. Williston Golden, Williston Brown, and Bombay are susceptible and Buda is moderately susceptible to race 24, but only Williston Golden and Williston Brown are susceptible to race 6.

The methods employed in selfing, hybridizing, and making pathogenicity determinations have been described previously (*5*). The studies were conducted in the pathology greenhouse at Fargo, N. Dak., during the winter months. Telia of race 22 were developed on Ottawa 770B, those of race 24 on Bombay, and those of race 6 on Williston Golden. Bison flax, a variety susceptible to each of these races, was subjected to sporidial inoculation. Hybridization was accomplished by transferring the pycnial nectar from a haploid infection of one race to a haploid infection of another. The pathogenicity of the uredial cultures was determined, and the $F_1$ telia were matured. The $F_2$ cultures were obtained by selfing the haploid pycnial infections developed from the sporidia produced by the $F_1$ teliospores. In selfing, precautions were taken so that pycniospores from one pycnium would effect fertilization of not more than one aecium.

Teliospore germination is capricious, and plans to backcross the $F_2$ hybrids with the parental cultures were not consummated, because of inability to obtain simultaneous germination of hybrid and parental teliospores.

The pathogenicity of each culture, based on infection types as previously described (*3*, *5*), was determined by the reaction of 16 differential varieties. Cultures inducing immune or resistant reactions were classed as avirulent, the immune reaction being considered as more highly avirulent than the resistant reaction. Cultures inducing susceptible reactions were classed as virulent, those inducing semiresistant reactions as semivirulent, and those inducing moderately susceptible reactions as moderately virulent. The $\chi^2$ test for goodness of fit (*2*) was used in analyzing the data.

## EXPERIMENTAL RESULTS

### CROSSES OF RACE 22 WITH RACE 24

In table 1 are given the reactions of the 16 rust-differentiating varieties of flax to parent races 22 and 24, to selfed cultures of the parent races, and to the $F_1$ and $F_2$ hybrid cultures. Also given in this table is the race number assigned to each culture based on the reactions

[4] B. Golden used by North Dakota Agricultural Experiment Station.

TABLE 1.—*Reaction of 16 rust-differentiating varieties of flax to races 22 and 24 of Melampsora lini, to selfed parental cultures, and to $F_1$ and $F_2$ cultures of hybrids of these races*

| Parent race, selfed parental race, or hybrid culture | Cultures having indicated pathogenicity | Reaction[1] of indicated differential variety | | | | | | | | | | | | | | | | Race No. assigned |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Buda | Williston Golden | Williston Brown | Akmolinsk | J.W.S. | Pale Blue Crimped[2] | Kenya | Abyssinian | Morye | Ottawa 770B | Bombay | Newland | Bolley Golden | Italia Roma | Leona | Tammes Pale Blue | |
| | *Number* | | | | | | | | | | | | | | | | | |
| Race 22 | 1 | S | S | S | S | I | S | S | S | S | S | I | S | S | S | S | S | 22 |
| Race 22, selfed | 9 | S | S | S | S | I | S | S | I | I | I | I | (3) | (3) | (3) | (3) | (3) | 22 |
| Race 24 | 1 | S– | S | S | R | I | R– | R | I | I | I | I | I | I | I | R | I | 24 |
| Race 24, selfed | 15 | S– | S | S | R | I | R– | R | I | I | I | I | I | I | I | R | I | 24 |
| $F_1$ hybrid, race 22 × race 24 | 7 | S | S | S | R | I | R– | R | I | I | I | I | I | I | I | R | I | 2 |
| | 1 | S | R | R | R | I | R– | R | I | I | I | I | I | I | I | R | I | 44 |
| | 1 | S– | R | R | R | I | R– | R | I | R | I | I | I | I | I | R | I | |
| | 3 | S– | R | R | R | I | R– | R | I | I | I | I | I | I | I | R | I | 45 |
| | 1 | S | R | R | R | I | R– | R | I | I | I | I | I | I | I | R | I | 46 |
| | 1 | S | R | R | R | I | R– | R | I | I | I | I | I | I | I | R | R | 47 |
| | 1 | S– | R | R | R | I | S | S | I | I | I | I | I | I | S– | R | I | 48 |
| | 2 | S | R | R | R | I | S | S | I | S– | I | I | I | R | I | R | S | 49 |
| | 1 | S– | R | R | R | I | S | S | I | I | I | I | I | I | I | R | S | 50 |
| | 1 | S– | R | R | R | I | R– | R | I | I | I | I | I | R | I | R | S | 51 |
| | 1 | S | R | R | R | I | R– | R | I | I | I | I | I | I | R | R | S | 52 |
| | 1 | S | R | R | R | I | R– | R | I | I | I | I | I | I | I | R | I | 53 |
| | 1 | S | R | R | R | I | R– | R | I | I | I | I | I | I | I | R | I | 54 |
| | 1 | S | R | R | R | I | R– | R | I | I | I | I | I | I | I | R | I | 55 |
| | 4 | S– | S | S | R | I | R– | R | I | I | I | I | I | I | I | R | I | |
| | 1 | S– | S | S | R | I | R– | R | I | I | I | I | I | I | I | R | I | |
| | 6 | S | S | S | R | I | R– | R | I | I | I | I | I | I | I | R | I | 2 |
| | 4 | S | S | S | R | I | R– | R | I | I | I | I | I | I | I | R | I | |
| | 1 | S | S | S | R | I | R– | R | I | I | I | I | I | I | I | R | I | |
| | 2 | S | S | S | R | I | R– | R | I | I | I | I | I | I | I | R | I | |
| | 5 | S | S | S | R | I | R– | R | I | I | I | I | I | I | I | R | I | |
| | 1 | S | S | S | R | I | R– | R | I | I | I | I | I | I | I | R | I | 56 |
| | 1 | S | S | S | R | I | R– | R | I | I | I | I | I | I | R | R | I | 57 |
| | 1 | S | S | S | R | I | R– | R | I | I | I | I | I | I | S | R | I | 58 |
| | 1 | S– | S | S | R | I | R– | R | I | I | I | I | I | I | S | R | I | 59 |
| | 1 | S– | S | S | R | I | R– | R | I | I | I | I | I | I | I | R | I | |
| | 1 | S– | S | S | R | I | R– | R | I | I | I | I | I | I | I | R | I | |
| | 1 | S– | S | S | R | I | R– | R | I | I | I | I | I | I | I | R | I | 60 |
| | 4 | S | S | S | R | I | R– | R | I | I | I | I | S | I | I | R | I | |
| | 1 | S | S | S | R | I | R– | R | I | I | I | I | S | I | I | R | I | |
| | 1 | S | S | S | R | I | R– | R | I | I | I | I | S | I | I | R | S | 61 |
| | 1 | S– | S | S | R | I | R– | R | I | I | I | I | S | I | S– | R | I | 62 |

See footnotes at end of table.

TABLE 1.—*Reaction of 16 rust-differentiating varieties of flax to races 22 and 24 of Melampsora lini, to selfed parental cultures, and to $F_1$ and $F_2$ cultures of hybrids of these races*—Continued

| Parent race, selfed parental race, or hybrid culture | Cultures having indicated pathogenicity | Reaction [1] of indicated differential variety | | | | | | | | | | | | | | | Race No. assigned |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Buda | Williston Golden | Williston Brown | Akmolinsk | J. W. S. | Pale Blue Crimped [2] | Kenya | Abyssinian | Morye | Ottawa 770B | Bombay | Newland | Bolley Golden | Italia Roma | Leona | Tammes Pale Blue | |
| | Number | | | | | | | | | | | | | | | | | |
| | 1 | S | S | S | S | I | R— | R | S | I | I | I | I | R | R | S | R | 96 |
| | 2 | S | S | S | S | I | R— | R | S | I | I | I | I | I | I | | I | |
| | 1 | S | S | S | S | I | R— | R | S | I | I | I | I | R | R | S | S | 97 |
| | 1 | S | S | S | S | I | R— | R | S | I | I | I | I | S | S | | I | 98 |
| | 2 | S | S | S | S | I | R— | R | S | I | I | I | I | I | I | | I | 99 |
| Fsegregates | 1 | S | S | S | S | I | R— | R | S | I | I | I | S | I | I | S | I | 100 |
| | 1 | S | S | S | S | I | R— | R | S | I | I | I | S | I | I | S | S | 101 |
| | 1 | S | S | S | S | I | R— | R | S | I | I | I | S | I | I | S | I | |
| | 1 | S | S | S | S | I | R— | R | S | I | I | I | S | I | I | S | I | 102 |
| | 1 | S | S | S | S | I | R— | R | S | I | I | I | S | I | I | S | I | |
| | 1 | S | S | S | S | I | R— | R | S | I | I | I | S | I | I | S | I | 103 |
| | 1 | S | S | S | S | I | S— | R | S | I | I | I | S | I | I | S | I | 104 |
| | 1 | S | S | S | S | I | S— | R | S | I | I | I | S | R | I | S | I | |
| | 3 | S | S | S | S | I | S | S | S | I | I | I | I | R | S— | S | I | 105 |
| Number of $F_2$ cultures | 133 | | | | | | | | | | | | | | | | | |
| Highly avirulent | | 0 | 0 | 0 | 0 | 133 | 0 | 0 | 110 | 121 | 101 | 105 | 97 | 100 | 97 | 0 | 76 | |
| Avirulent | | 0 | 17 | 17 | 110 | 0 | 96 | 96 | 0 | 2 | 0 | 0 | 0 | 27 | 17 | 110 | 22 | |
| Moderately virulent | | 39 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 10 | 0 | 0 | |
| Virulent | | 94 | 116 | 116 | 23 | 0 | 37 | 37 | 23 | 3 | 32 | 28 | 36 | 6 | 9 | 23 | 35 | |

[1] The letters signify the following host reactions: I, Immune; R, resistant; and S, susceptible. Minus signs indicate somewhat less resistance or susceptibility than letters alone.

[2] The reaction of Pale Blue Crimped to avirulent cultures is influenced by environment and in these studies varied from resistant to moderately susceptible. Variations attributable to environment have been disregarded, and this variety has been classed as moderately resistant to all avirulent cultures.

[3] Not in use as differentials when original tests were made. These varieties were susceptible, however, to a specimen culture maintained for reference.

[4] Not in use as differentials when original tests were made. These varieties reacted, however, to a specimen culture maintained for reference as to the race 24 parent.

of the differential varieties. It will be noted that the pathogenicity tests of selfed cultures of races 22 and 24 indicate that these races are pure. However, the number of selfed cultures tested was too small to be certain that each was homozygous, especially if virulence on a particular variety was conditioned by dominant factors or by more than 1 pair of recessive factors.

Irrespective of which way the cross was made, the $F_1$ hybrid cultures were identical in pathogenicity. Infection of the virulent type appeared to be a recessive character, since $F_1$ cultures were unable to attack varieties resistant to either parent race. The reaction of Buda to the $F_1$ cultures was intermediate to its reaction to the parent races; it was more susceptible to the $F_1$ cultures than to race 24 but somewhat less susceptible than to race 22. Varieties susceptible to both parent races were susceptible to the $F_1$ cultures.

Of the 133 $F_2$ cultures studied, 49 were from an $F_1$ culture resulting from transfer of pycniospores of race 22 to a haploid pustule of race 24 and 84 were from an $F_1$ culture obtained by transferring pycniospores of race 24 to a haploid pustule of race 22. Since there was no indication that the reciprocal crosses differed in pathogenicity the data have been combined. These 133 $F_2$ cultures comprised 64 physiologic races, of which 62 had not been found in nature. The 64 races were differentiated from one another by sharp differences in pathogenicity on 1 or more varieties of flax. A difference between an immune and a resistant or between a moderately susceptible and a susceptible host reaction is considered insufficient to differentiate races.

Little significance should be attached to the difference between the immune reaction of Abyssinian and the resistant reactions of Kenya and Leona. Under conditions favorable for rust development small, usually subepidermal pustules are often formed in the heavily inoculated portions of the leaves of the last two, whereas similar pustules are rarely formed on Abyssinian. Under less favorable conditions, the reactions of all three varieties are frequently read as immune. However, the reaction of Abyssinian is definitely resistant to some races attacking Akmolinsk; numerous small pustules producing appreciable numbers of spores are formed even under rather adverse conditions.

On the other hand, the difference between the resistant and immune reaction of Bolley Golden and Italia Roma is sufficiently distinct to be used as a criterion for separating races. However, the difference between an immune and a resistant reaction is of little practical signifiance and races have not been differentiated on this basis. Varieties classed as resistant in the seedling stage in greenhouse tests have been practically immune when exposed to the same races under field conditions.

The ratios of $F_2$ segregates that were virulent to those that were avirulent on each of the 16 rust-differentiating varieties of flax are given in table 2.

TABLE 2.—*Segregation of $F_2$ cultures of a cross of race 22 with race 24 of Melampsora lini for pathogenicity on 16 rust-differentiating varieties of flax*

| Variety | Pathogenicity of— | | | | | | $\chi^2$ value [1] |
|---|---|---|---|---|---|---|---|
| | Parent race | | $F_1$ | $F_2$ segregates | | | |
| | | | | Observed | | Theoretical ratio (virulent to avirulent) | |
| | 22 | 24 | | Virulent | Avirulent | | |
| | | | | *Number* | *Number* | | |
| Buda | Virulent | Virulent | Virulent | 133 | 0 | 1:0 | |
| Williston Golden [2] | do | do | do | 116 | 17 | 3:1 | 10.59 |
| | | | | | | 15:1 | 9.69 |
| Williston Brown | | | | | | | |
| J. W. S | Avirulent | Avirulent | Avirulent | 0 | 133 | 0:1 | |
| Akmolinsk [2] | Virulent | do | do | 23 | 110 | 1:3 | 4.21 |
| Abyssinian | | | | | | | |
| Leona | | | | | | | |
| Pale Blue Crimped [2] | do | do | do | 37 | 96 | 1:3 | .55 |
| Kenya | | | | | | | |
| Ottawa 770B | do | do | do | 32 | 101 | 1:3 | .06 |
| Bombay | Avirulent | Virulent | do | 28 | 105 | 1:3 | 1.07 |
| Newland | Virulent | Avirulent | do | 36 | 97 | 1:3 | .30 |
| Tammes Pale Blue | do | do | do | 35 | 98 | 1:3 | .12 |
| Bolley Golden | do | do | do | 6 | 127 | 1:15 | .69 |
| Italia Roma | do | do | do | 9 | 124 | [3] 1:15 | .06 |
| Morye | do | do | do | 3 | 130 | [3] 1:63 | .41 |

[1] 5-percent point=3.84; 1-percent point=6.64.

[2] Pathogenicity on varieties in each group inherited as a unit.

[3] Italia Roma and Morye were moderately susceptible to some $F_2$ cultures, indicating that 1 of the 2 pairs of factors conditioning avirulence of race 24 on the former and 1 of the 3 pairs of factors conditioning avirulence on the latter are incompletely dominant and may be quantitative, heterozygous cultures being more virulent than homozygous dominant ones. Only the $F_2$ cultures to which these varieties are completely susceptible are homozygous for the recessive factors of the race 22 parent for virulence and have been classed as virulent in the ratio of virulent to avirulent segregates.

The pathogenic characters for which the $F_2$ cultures segregated were analyzed for independent assortment in all possible combinations of pairs by use of the $\chi^2$ test. These $\chi^2$ values (table 3) are based on the assumption that in the cross of race 22 with race 24 single-factor pairs condition pathogenicity on Williston Golden, Williston Brown, Akmolinsk, Abyssinian, Leona, Pale Blue Crimped, Kenya, Ottawa 770B, Bombay, Newland, and Tammes Pale Blue; that two-factor pairs condition pathogenicity on Bolley Golden and Italia Roma; and that three-factor pairs condition pathogenicity on Morye. In analyzing the data it is assumed that a $P$ value greater than 0.05 indicates lack of association of the factors conditioning pathogenicity on the paired varieties.

TABLE 3.—$\chi^2$ *values for independent assortment of pathogenic factors in the* $F_2$ *progeny of a cross of race 22 with race 24 of Melampsora lini*

| Differential varieties grouped according to pathogenicity assumed to be conditioned by recessive factors | Recessive $F_1$ cultures | $\chi^2$ value [1] for— | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Avirulent (1 pair of recessive factors) | Virulent with— | | | | | | | | |
| | | | 1 pair of recessive factors | | | | | | 2 pairs of recessive factors | | 3 pairs of recessive factors |
| | | Williston Golden and Williston Brown | Akmolinsk, Abyssinian, and Leona | Pale Blue Crimped and Kenya | Ottawa 770B | Bombay | Newland | Tammes Pale Blue | Bolley Golden | Italia Roma | Morye |
| Avirulent (1 pair of recessive factors): | | | | | | | | | | | |
| Williston Golden [2] | 17 | ------ | 0.18 | 0.18 | 0.96 | 0.18 | 1.59 | 0.02 | 1.13 | 1.13 | 2.00 |
| Williston Brown | | | | | | | | | | | |
| Virulent (1 pair of recessive factors): | | | | | | | | | | | |
| Akmolinsk [2] | 23 | 0.13 | ------ | .13 | .36 | .71 | .36 | .13 | .14 | 4.85 | .37 |
| Abyssinian | | | | | | | | | | | |
| Leona | | | | | | | | | | | |
| Pale Blue Crimped [2] | 37 | 2.60 | 2.60 | ------ | 1.52 | .73 | .73 | .44 | .44 | 6.29 | .31 |
| Kenya | | | | | | | | | | | |
| Ottawa 770B | 32 | .67 | .17 | .67 | ------ | 1.50 | 1.50 | 0 | 2.13 | 2.13 | .51 |
| Bombay | 28 | .76 | 1.72 | 0 | .76 | ------ | .19 | .76 | .34 | 1.87 | .72 |
| Newland | 36 | 7.26 | .59 | .59 | .59 | .15 | ------ | 0 | .74 | .03 | .35 |
| Tammes Pale Blue | 35 | 3.44 | 2.14 | .77 | .09 | .01 | .01 | ------ | .02 | .69 | 11.20 |
| Virulent (2 pairs of recessive factors): | | | | | | | | | | | |
| Bolley Golden | 6 | 2.00 | .22 | .22 | 2.00 | .22 | .22 | .23 | ------ | 7.51 | .10 |
| Italia Roma | 9 | 3.00 | 1.81 | 8.33 | 3.00 | 3.00 | .04 | .02 | 3.95 | ------ | .14 |
| Virulent (3 pairs of recessive factors): | | | | | | | | | | | |
| Morye | 3 | .11 | 1.00 | .11 | 1.00 | 2.78 | .11 | 9.00 | .20 | .20 | ------ |

[1] 5-percent point=3.84; 1-percent point=6.64.
[2] Pathogenicity on varieties in each group inherited as a unit.

### CROSSES OF RACE 6 WITH RACE 22

Viable teliospores were obtained from 3 $F_1$ cultures of hybrids of race 6 with race 22, the pathogenicity of the $F_2$ cultures was studied, and the results are presented in table 4. Pathogenicity tests of selfed parental and $F_1$ hybrid cultures indicated (*5*) that race 22 was homozygous and that race 6 was heterozygous for pathogenicity on Akmolinsk, avirulence being dominant and conditioned by a single factor. Akmolinsk was susceptible to the $F_1$ culture of hybrid A, and each of the 74 $F_2$ cultures was virulent on Akmolinsk. The $F_1$ cultures of hybrid B and hybrid C were avirulent on Akmolinsk, and the $F_2$ cultures of each segregated for virulence on this variety.

TABLE 4.—*Reaction of 16 rust-differentiating flax varieties to races 6 and 22 of Melampsora lini, to selfed parental cultures, and to $F_1$ and $F_2$ cultures of hybrids of these races*

| Parent race, selfed parental race, or hybrid culture | Cultures having indicated pathogenicity | Reaction[1] of indicated differential variety | | | | | | | | | | | | | | | | Race No. assigned |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Buda | Williston Golden | Williston Brown | Akmolinsk | J.W.S. | Pale Blue Crimped[2] | Kenya | Abyssinian | Morye | Ottawa 770B | Bombay | Newland | Bolley Golden[2] | Italia Roma[2] | Leona | Tammes Pale Blue[2] | |
| | *Number* | | | | | | | | | | | | | | | | | |
| Race 6 | 1 | R | S | S | R | I | R– | R | I | I | I | I | I(-) | I(-) | I(-) | R(-) | I(-) | 6 |
| Race 6, selfed | 7 | R | S | S | R | I | R– | R | I | I | I | I | I(-) | I(-) | I(-) | R(-) | I(-) | 6 |
| Do | 2 | R | S | S | S | I | R– | R | R | I | I | I | S(-) | S(-) | S(-) | S(-) | S(-) | 33 |
| Race 22 | 1 | S | S | S | S | I | S | S | S | I | S | I | I(-) | I(-) | I(-) | I(-) | I(-) | 22 |
| Race 22, selfed | 9 | S | S | S | S | I | S | S | S | S | S | I | I(-) | I(-) | I(-) | I(-) | I(-) | 22 |
| Hybrid A, race 6 × race 22: $F_1$ culture | 1 | SR | S | S | S | I | R– | R | R | I | I | I | I | I | I | R | I | 34 |
| $F_2$ segregates | 2 | R | S | S | S | I | R– | R | R | I | I | I | I | R | I | R | I | 33 |
| | 1 | R | S | S | S | I | R– | R | R | I | I | I | I | I | I | R | I | 106 |
| | 1 | R | S | S | S | I | R– | R | R | I | I | I | I | I | I | R | I | 107 |
| | 1 | R | S | S | S | I | R– | R | R | I | I | I | I | I | I | R | I | 108 |
| | 1 | R | S | S | S | I | R– | R | R | I | I | I | I | I | I | R | I | 109 |
| | 2 | R | S | S | S | I | R– | R | S | I | I | I | I | I | I | R | I | 110 |
| | 2 | R | S | S | S | I | S | S | R | I | I | I | I | I | I | R | I | 111 |
| | 1 | R | S | S | S | I | S | S | R | I | I | I | I | I | I | R | I | 34 |
| | 19 | SR | S | S | S | I | R– | R | R | I | I | I | I | R | I | R | I | 112 |
| | 6 | SR | S | S | S | I | R– | R | R | I | I | I | I | S | I | R | I | 113 |
| | 3 | SR | S | S | S | I | R– | R | R | I | I | I | I | I | I | R | I | 114 |
| | 6 | SR | S | S | S | I | R– | R | R | I | I | I | I | R | I | R | I | 115 |
| | 1 | SR | S | S | S | I | R– | R | R | I | I | I | I | I | I | R | (-) | 116 |
| | 3 | SR | S | S | S | I | R– | R | R | I | I | I | I | R | I | R | I | 117 |
| | 1 | SR | S | S | S | I | R– | R | R | I | I | I | I | I | I | R | I | 118 |
| | 1 | SR | S | S | S | I | R– | R | R | I | I | I | I | I | I | S | I | 119 |
| | 2 | SR | S | S | S | I | R– | R | S | I | I | I | I | S | I | S | I | 120 |
| | 1 | SR | S | S | S | I | R– | R | S | I | I | I | I | I | I | S | I | 121 |
| | 1 | SR | S | S | S | I | R– | R | S | I | I | I | I | R | I | R | I | 122 |
| | 4 | SR | S | S | S | I | S | S | S | I | I | I | I | R | I | R | I | 123 |
| | 2 | SR | S | S | S | I | S | S | S | I | I | I | I | S | I | R | I | 124 |
| | 2 | SR | S | S | S | I | S | S | S | I | I | I | I | S | I | R | I | 125 |
| | 1 | SR | S | S | S | I | S | S | S | I | I | I | I | S | I | S | I | 126 |
| | 1 | SR | S | S | S | I | S | S | S | I | I | I | I | I | I | S | I | 127 |
| | 1 | SR | S | S | S | I | S | S | S | I | I | I | I | S | I | S | I | 128 |
| | 1 | SR | S | S | S | I | S | S | S | I | I | I | I | S | I | S | I | 96 |
| | 3 | S | S | S | S | I | R– | R | R | I | I | I | I | S | I | S | I | 129 |
| | 1 | S | S | S | S | I | R– | R | R | I | I | I | I | I | I | S | I | 90 |
| | 2 | S | S | S | S | I | R– | R | R | I | I | I | I | I | I | S | I | 100 |
| | 1 | S | S | S | S | I | R– | R | S | I | I | I | S | I | I | S | I | 102 |

| | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Number of virulent cultures observed | | 8 | 74 | 74 | 74 | 0 | 15 | 15 | 17 | 0 | 10 | 0 | 17 | 6 | 18 | 17 | 5 | |
| Number of virulent cultures expected | | 4.6 | 74 | 74 | 74 | 0 | 18.5 | 18.5 | 18.5 | 1.2 | 18.5 | 0 | 18.5 | 4.6 | 18.5 | 18.5 | 4.6 | |
| Theoretical ratio: virulent to avirulent cultures | | 1:15 | | | | | 1:3 | 1:3 | 1:3 | 1:63 | 1:3 | | 1:3 | 1:15 | 1:3 | 1:3 | 1:15 | |
| $x^2$ value [7] | | 2.68 | | | | | .88 | .88 | .16 | 1.17 | 5.21 | | .16 | .45 | .02 | .16 | .04 | |
| **Hybrid B, race 22 × race 6:** | | | | | | | | | | | | | | | | | | |
| $F_1$ culture | 1 | SR | S | S | R | I | R− | R | I | I | I | I | I | I | I | R | I | 14 |
| $F_2$ segregates | 1 | R | S | S | R | I | S | S | I | I | I | I | S | I | I | R | I | 130 |
| | 5 | SR | S | S | R | I | R− | R | I | I | I | I | I | I | I | R | I | 14 |
| | 1 | SR | S | S | R | I | R− | R | I | I | I | I | I | I | S− | R | I | 131 |
| | 2 | SR | S | S | R | I | R− | R | I | I | I | I | S | I | I | R | I | 132 |
| | 1 | SR | S | S | R | I | R− | R | I | I | S | I | I | I | I | R | I | 133 |
| | 3 | SR | S | S | R | I | S | S | I | I | I | I | I | I | I | R | I | 134 |
| | 1 | S− | S | S | R | I | R− | R | I | I | I | I | I | I | I | R | I | 2 |
| | 1 | S | S | S | S | I | R− | R | S | I | I | I | I | I | I | S | I | 96 |
| | 1 | S | S | S | S | I | R− | R | S | I | I | I | S | I | I | S | S | 100 |
| **Hybrid C, race 6 × race 22:** | | | | | | | | | | | | | | | | | | |
| $F_1$ culture | 1 | SR | S | S | R | I | R− | R | I | I | I | I | I | I | I | R | I | 14 |
| $F_2$ segregates | 1 | R | S | S | R | I | R− | R | I | I | I | I | (8) | (8) | (8) | (8) | (8) | 6 |
| | 3 | SR | S | S | R | I | R− | R | I | I | I | I | (8) | (8) | (8) | (8) | (8) | 14 |
| | 2 | SR | S | S | R | I | R− | R | I | I | S | I | (8) | (8) | (8) | (8) | (8) | 133 |
| | 1 | S− | S | S | R | I | R− | R | I | I | I | I | (8) | (8) | (8) | (8) | (8) | 2 |
| | 1 | S | S | S | S | I | R− | R | S | I | I | I | I | R | S | S | I | 135 |

[1] The letters signify the following host reactions: I, Immune; R, resistant; SR, semi-resistant; and S, susceptible. Minus signs indicate somewhat less resistance or susceptibility than letters alone.

[2] The reaction of Pale Blue Crimped to avirulent cultures is influenced by environment and in these studies varied from resistant to moderately susceptible. Variations attributable to environment have been disregarded, and this variety has been classed as moderately resistant to all avirulent cultures.

[3] Bolley Golden, Italia Roma, and Tammes Pale Blue, resistant to some $F_2$ cultures, are here classed as immune.

[4] Not in use as differentials when these tests were made. These varieties reacted, however, to a specimen culture maintained for reference as to the race 6 parent.

[5] Not in use as differentials when these tests were made. These varieties were susceptible, however, to a specimen culture maintained for reference.

[6] Accidentally lost before reaction was determined; not retested.

[7] 5-percent point=3.84; 1-percent point=6.64.

[8] Not in use as differentials when these tests were made.

The selfed parental cultures and all but 1 of the $F_2$ cultures of hybrid C had been tested and discarded before the differentiating potentialities of Newland, Bolley Golden, Italia Roma, Leona, and Tammes Pale Blue were ascertained. Among the 74 $F_2$ cultures of hybrid A, 30 races were identified. The 16 $F_2$ cultures of hybrid B yielded 9 races, and the 8 $F_2$ cultures of hybrid C yielded 5 races. Thirty-six races that had not been isolated from any field collection were obtained from the 98 $F_2$ cultures of these crosses. Because the parental gametes of race 6 were heterozygous, the data could not be combined for statistical analysis and an insufficient number of cultures of each hybrid was tested to warrant an analysis of the interaction of factors for pathogenicity similar to that made of $F_2$ cultures of hybrids between races 22 and 24.

## GENOTYPES OF PARENTAL RACES

Letters were used to designate pathogenic factors in the flax rust fungus in an earlier publication (*5*). However, the numerous factors that have now been demonstrated to condition pathogenicity make it desirable to use a more flexible system of designation. In table 5 are presented the suggested genotypes of parent races 6, 22, and 24 of *Melampsora lini* as indicated by the segregation for virulence on 15 differential flax varieties of $F_2$ cultures of hybrids of these races. The symbol *A* designates the dominant avirulent and *a* the recessive virulent. The dominant factor conditioning virulence on Williston Golden and Williston Brown is designated by *V* and its recessive allelomorph by *v*. Characteristic letters designate the variety on which each factor conditions pathogenicity.

Because the interaction between the factors for pathogenicity in some races of *Melampsora lini* with the factors for rust reaction in Buda is quantitative and is subject to considerable influence by environment, it is necessary to exercise rigid control of the conditions under which the tests are made in order to differentiate the genotypes accurately. Since the difference between the virulence of race 22 and the moderate virulence of race 24 on Buda is a minor one, the information obtainable from a study of the $F_2$ hybrids of a cross between these two races (table 1) did not warrant the elaborate precautions required to differentiate genotypes accurately. The occurrence of 39 moderately virulent to 94 virulent cultures on Buda satisfactorily approximates the 1:3 ratio expected if race 22 carried 2 pairs of recessive factors conditioning virulence on Buda and race 24 carried 1 pair conditioning virulence and a second pair conditioning moderate virulence. Because of the quantitative relation between the factors for pathogenicity in some races of *M. lini* and the factors for reaction to rust in Buda, it is possible that the second pair of factors may be heterozygous in some of the $F_2$ cultures classed as moderately virulent. Consequently, no statistical analysis has been made of the interaction of the factors for pathogenicity on Buda with those for pathogenicity on the other varieties.

TABLE 5.—*Suggested genotypes of races 6, 22, and 24 of Melampsora lini and factors conditioning pathogenicity on 15 differential flax varieties*

[The letters in "Reaction" columns signify the following host reactions: I, Immune; R, resistant; and S, susceptible. Minus signs indicate somewhat less resistance or susceptibility than letters alone]

| Differential variety | Race 6 [1] | | | Race 22 [2] | | Race 24 [3] | | |
|---|---|---|---|---|---|---|---|---|
| | Genotype conditioning pathogenicity on indicated variety | Reaction | Previously used symbols (*b*) | Genotype conditioning pathogenicity on indicated variety | Reaction | Genotype conditioning pathogenicity on indicated variety | Reaction | Previously used symbols (*b*) |
| Buda | $A_{bu}A_{bu}A_{bu}A_{bu}A_{bu}$ | R | *AABB* | $G_{bu}G_{bu}G_{bu}G_{bu}G_{bu}$ | S | $G_{bu}G_{bu}G_{bu}{}^{-1}G_{bu}{}^{-1}$ | S− | *aabb.* |
| Akmolinsk | $A_{ak}G_{ak}$ | R | *Cc* | $G_{ak}G_{ak}$ | S | $A_{ak}A_{ak}$ | R | *CC.* |
| Abyssinian | $A_{ab}A_{ab}$ | I | | $G_{ab}G_{ab}$ | S | $A_{ab}A_{ab}$ | I | |
| Leona | $A_{le}A_{le}$ | R | | $G_{le}G_{le}$ | S | $A_{le}A_{le}$ | R | |
| Bombay | $A_{bom}A_{bom}$ | I | *DD* | $A_{bom}A_{bom}$ | S | $G_{bom}G_{bom}$ | S | *dd.* |
| Williston Golden<br>Williston Brown | $V_{wb}V_{wb}$ | S | | $V_{wb}V_{wb}$ | S | $V_{wb}V_{wb}$ | S | |
| Pale Blue Crimped | $A_{p}A_{p}$ | R− | | $G_{p}G_{p}$ | S | $A_{p}A_{p}$ | R− | |
| Kenya | $A_{k}A_{k}$ | R | | $G_{k}G_{k}$ | S | $A_{k}A_{k}$ | R | |
| Ottawa 770B | $A_{o}A_{o}$ | I | | $G_{o}G_{o}$ | S | $A_{o}A_{o}$ | I | |
| Newland | $A_{n}A_{n}$ | I | | $G_{n}G_{n}$ | S | $A_{n}A_{n}$ | I | |
| Tammes Pale Blue | $A_{tpb}A_{tpb}A_{tpb}A_{tpb}$ | I | | $G_{tpb}G_{tpb}G_{tpb}G_{tpb}$ | S | $A_{tpb}A_{tpb}G_{tpb}G_{tpb}$ | I | |
| Bolley Golden | $A_{bg}A_{bg}A_{bgl}A_{bgl}$ | I | | $G_{bg}G_{bg}G_{bgl}G_{bgl}$ | S | $A_{bg}A_{bg}A_{bgl}A_{bgl}$ | I | |
| Italia Roma | $A_{ir}A_{ir}A_{ir}A_{ir}$ | I | | $G_{ir}G_{ir}G_{ir}G_{ir}$ | S | $A_{ir}A_{ir}A_{ir}A_{ir}$ | I | |
| Morye | $A_{tpb}A_{tpb}A_{arl}A_{arl}A_{arl}A_{arl}$ | I | | $G_{tpb}G_{tpb}G_{arl}G_{arl}G_{arl}G_{arl}$ | S | $A_{tpb}A_{tpb}A_{arl}A_{arl}{}^{-1}G_{arl}{}^{-1}$ | I | |

[1] Genotype for race 6: $A_{bu}A_{bu}A_{bu}A_{ak}A_{ak}A_{le}A_{tpbl}/A_{bu}{}^{1}G_{ak}A_{ak}A_{le}A_{tpbl}A_{bom}A_{bom}V_{wb}V_{wb}A_{p}A_{k}A_{ir}/A_{p}A_{k}G_{ir}A_{o}A_{o}A_{n}A_{n}A_{bg}A_{bg}A_{bgl}A_{bgl}A_{tpb}A_{tpb}A_{arl}A_{arl}$.

[2] Genotype for race 22: $G_{bu}G_{bu}G_{bu}{}^{1}G_{ak}{}^{1}G_{ak}{}^{1}G_{le}G_{tpbl}/G_{bu}{}^{1}G_{ak}{}^{1}G_{ak}{}^{1}G_{le}G_{tpbl}A_{bom}A_{bom}V_{wb}V_{wb}G_{p}{}^{2}G_{k}{}^{2}G_{ir}G_{o}G_{o}G_{n}G_{n}G_{bg}G_{bg}G_{bgl}G_{bgl}G_{tpb}G_{tpb}G_{arl}G_{arl}G_{arl}G_{arl}$.

[3] Genotype for race 24: $G_{bu}G_{bu}G_{bu}{}^{-1}A_{ak}A_{ak}A_{le}G_{tpbl}/G_{bu}{}^{-1}A_{ak}A_{ak}A_{le}G_{tpbl}G_{bom}G_{bom}V_{wb}V_{wb}A_{p}A_{k}A_{ir}/A_{p}A_{k}A_{ir}A_{o}A_{o}A_{n}A_{n}A_{bg}A_{bg}A_{bgl}A_{bgl}A_{tpb}A_{tpb}A_{arl}A_{arl}{}^{-1}G_{arl}{}^{-1}$.

### Pathogenicity on Buda

The inheritance of pathogenicity on Buda was adequately explored in the study on the hybrids of race 6 with race 24 (*5*) and explained by assuming that two pairs of factors were involved. When both dominant factors were homozygous the avirulence of the race 6 parent was obtained, and when both recessive factors were homozygous the moderate virulence of the race 24 parent was obtained. When either factor or both were heterozygous, pathogenicity was intermediate.

The reaction of Buda to the 98 $F_2$ cultures of the hybrids of race 6 with race 22 (table 4) ranged from resistant to susceptible, agreeing with prior studies (*5*) of a hybrid between a virulent and an avirulent race. Since the elaborate precautions required to determine accurately the genotypes for pathogenicity on Buda were not taken, a detailed statistical analysis was not attempted. Eight cultures of hybrid A that were virulent on Buda may not have been homozygous for the race 22 factors for virulence on this variety, but even if they were, the ratio does not deviate significantly from the 1:15 ratio expected.

In the cross of race 6 with race 24, linkage was observed between one of the factors for avirulence on Buda and the factor for virulence on Akmolinsk (*5*). As shown in table 1, linkage between these same factors or their allelomorphs persists in the cross of race 22 with race 24. As in the previous study, this linkage is not complete, since Buda was highly susceptible to but 21 of the 23 cultures attacking Akmolinsk. Tests of the $F_2$ cultures of the hybrids of race 6 with race 22 (table 4) also show linkage between the factors for pathogenicity on Buda and Akmolinsk. In all hybrids involving race 22, the race 22 factors for virulence on Akmolinsk, Abyssinian, and Leona have been inherited as a unit. These 3 varieties are susceptible to each of the 11 $F_2$ cultures of race 6 $\times$ race 22 virulent on Buda, confirming the linkage of factors for pathogenicity on these varieties.

The segregation of the factors for pathogenicity on Buda may be explained by assuming two series of allelomorphic factors. Race 6 is homozygous for the incompletely dominant factors $A_{bu}$ and $A_{bu'}$ conditioning avirulence. In race 24 one factor, $a_{bu}$, conditions virulence and the other, $a_{bu'-1}$, linked with the factors for pathogenicity on Akmolinsk, Abyssinian, and Leona, conditions moderate virulence. In race 22 both factors, $a_{bu}$ and $a_{bu'}$, condition virulence.

### Pathogenicity on Williston Golden and Williston Brown

Williston Golden possesses 2 and Williston Brown 1 factor conditioning resistance to certain races of *Melampsora lini*, the factor in Williston Brown apparently being identical with 1 of the factors in Williston Golden (*6*). Since but 1 rust-conditioning factor apparently occurs in Williston Brown, this factor has been designated the Williston Brown factor. The second factor of Williston Golden, not possessed in common with Williston Brown, has been designated the Williston Golden factor. Both Williston Golden and Williston Brown are susceptible to the parent races used in these studies and to the selfed and $F_1$ hybrid cultures of these races. To 17 of 133 $F_2$ cultures of the cross of race 22 with race 24 (table 1) these varieties were resistant, pathogenicity on them apparently being inherited as a unit. As

shown in table 2, this distribution is not satisfactorily explained by assuming avirulence on Williston Golden and Williston Brown to be conditioned by either 1 or 2 pairs of recessive factors.

Several things may have contributed to the deficiency in the number of cultures avirulent on Williston Golden and Williston Brown. Because both parent races and all selfed and $F_1$ hybrid cultures were virulent on these varieties, the occurrence of avirulent $F_2$ cultures was not anticipated. Multiple-sporidial infections of the same sex cannot be accurately differentiated from single-sporidial infections; therefore it is possible that in some multiple infections the avirulent culture was not differentiated. Likewise, in a mechanical mixture of races the avirulent one might escape detection. Since all experimental errors would tend to reduce the number of avirulent cultures isolated, added significance is attached to the excellent fit of most of the observed to the expected ratios as indicated by the $\chi^2$ values (table 3) for independent assortment of pathogenic factors, assuming that avirulence on Williston Golden and Williston Brown is conditioned by one pair of recessive factors, $v_{w5}v_{w2}$.

The avirulence of the $F_2$ cultures of the race 22 × race 24 hybrids may have been due to mutation or to heterozygosity in one of the parent races of the factor conditioning pathogenicity on Williston Brown. Since avirulent cultures occurred in approximately equal ratios in both the race 22 × race 24 and the race 24 × race 22 progenies, the mutation theory is improbable. The data give little indication as to which race was heterozygous. Nine selfed cultures of race 22 and 15 of race 24 were tested. Both progenies of the hybrids of race 22 with race 24 yielded avirulent cultures, whereas none were obtained from the 3 progenies of hybrids of race 6 with race 22. Since Williston Golden and Williston Brown were somewhat more susceptible to race 22 than to race 24, the heterozygous factor has been tentatively assigned to the latter race.

No information as to the inheritance of pathogenicity on the Williston Golden factor for rust resistance is obtainable from these crosses, because all parent races apparently were homozygous for the factors conditioning virulence on it.

### Pathogenicity on Akmolinsk, Abyssinian, and Leona

In the cross of race 22 and race 24, virulence on Akmolinsk, Abyssinian, and Leona was inherited as a unit on a single-factorial basis and linked with one of the factors for virulence on Buda (table 1). This relation in virulence also held in the cross of race 6 with race 22 (table 4) except that in hybrid A all $F_2$ cultures were virulent on Akmolinsk. Parent race 6 was heterozygous for virulence on Akmolinsk, and the race 6 parental gamete of hybrid A apparently contained the recessive allelomorph conditioning virulence on this variety. On Akmolinsk, Abyssinian, and Leona avirulence apparently is completely dominant, as the heterozygous cultures cannot be distinguished from the homozygous ones.

Although virulence on Akmolinsk, Abyssinian, and Leona was inherited as a unit in the cross of race 22 with race 24 and in hybrid B and hybrid C of the cross of race 6 with race 22, it is necessary to assume a factor for pathogenicity on Akmolinsk distinct from those

on Abyssinian and Leona in order to explain the pathogenicity of hybrid A (table 4). Since Abyssinian is susceptible to a number of North American races to which Leona is resistant, these two varieties possess different rust-conditioning factors. In all instances analyzable from these crosses as well as from the hybrid of race 6 with race 24 (*5*) there is for each rust-conditioning factor in the host a factor for pathogenicity in the rust organism. Consequently, it is probable that separate but closely linked factors condition pathogenicity on Abyssinian and Leona. These studies indicate that race 6 was heterozygous for the factor for virulence on Akmolinsk and homozygous for the dominant factors conditioning avirulence on Abyssinian and Leona, $A_{ak}a_{ak}A_{ab}A_{ab}A_{le}A_{le}$; that race 24 was homozygous for the three pairs of dominant factors conditioning avirulence on Akmolinsk, Abyssinian, and Leona, $A_{ak}A_{ak}A_{ab}A_{ab}A_{le}A_{le}$; and that race 22 was homozygous for the recessive allelomorphs, $a_{ak}a_{ak}a_{ab}a_{ab}a_{le}a_{le}$, conditioning virulence on each of these varieties.

### Pathogenicity on Pale Blue Crimped and Kenya

Pathogenicity on Pale Blue Crimped and Kenya was inherited as a single factor with avirulence dominant (tables 1, 2, and 4). However, these varieties react quite differently to most races, and each differentiates naturally occurring physiologic races. Seedlings of Pale Blue Crimped have been moderately resistant to most North American races, whereas those of Kenya have been resistant to immune (*3*). Straib (*9*) found Kenya to be immune from eight European races, whereas the reaction of Pale Blue Crimped to these races ranged from immunity to moderate susceptibility. Since in all known instances there is a separate pathogenicity factor in *Melampsora lini* for each factor for resistance in flax, the factors conditioning pathogenicity on Pale Blue Crimped and Kenya are considered to be closely linked rather than identical. Consequently, it is assumed that races 6 and 24 carried two pairs of dominant factors, one, $A_pA_p$, conditioning avirulence on Pale Blue Crimped and the other, $A_kA_k$, avirulence on Kenya, and that race 22 carried the recessive allelomorphs, $a_pa_pa_ka_k$, conditioning virulence.

The analysis for independent assortment of pathogenic factors by means of the $\chi^2$ test (table 3) shows that the factors for pathogenicity on Pale Blue Crimped and Kenya are inherited independently of those for pathogenicity to all other differential varieties except Italia Roma.

Although the data clearly indicate that pathogenicity on Pale Blue Crimped and Kenya is conditioned by single major factors, variations in the reaction of these varieties, especially that of Kenya, suggest that one or more secondary factors may have been involved. This phase of the problem has not been investigated.

### Pathogenicity on Ottawa 770B

Ottawa 770B is an ideal differential in that it possesses a single rust-conditioning factor and has been either immune from or highly susceptible to all physiologic races with which it has been tested (*6*). As shown in table 2, the segregation of the $F_2$ cultures of the hybrid between races 22 and 24 for pathogenicity on Ottawa 770B

approximated the ratio of 1 virulent to 3 avirulent, assuming pathogenicity on this variety to be conditioned by a single factor. In the cross of race 6 with race 22 (table 4) there is a deficiency in the number of $F_2$ cultures virulent on Ottawa 770B. Because of the limited number of cultures tested and the close fit of the observed to the theoretical ratio in the race 22 × race 24 hybrids this deficiency probably is not significant. It is assumed that races 6 and 24 carry the dominant factors $A_oA_o$ conditioning avirulence on Ottawa 770B and that race 22 carries the recessive allelomorphs $a_oa_o$.

The analysis for independent assortment of pathogenic factors (table 3) indicates that the factor for pathogenicity on Ottawa 770B is inherited independently of all other factors for pathogenicity identifiable in this study.

### Pathogenicity on Bombay

Bombay, like Ottawa 770B, possesses a single pair of rust-conditioning factors (*6*) and has been either immune from or highly susceptible to each race of *Melampsora lini* with which it has been tested. The $F_2$ cultures of race 22 × race 24 segregated for pathogenicity on Bombay in a ratio indicating that a single pair of factors, $A_{bom}A_{bom}$, was involved, avirulence being dominant (tables 1 and 2). This confirms the study on the inheritance of pathogenicity on this variety in the hybrid of race 6 with race 24 (*5*). As indicated in table 3, the factor for virulence on Bombay is inherited independently of the factors conditioning virulence on the other differential varieties.

Bombay, immune from races 6 and 22, was immune from each of the 98 $F_2$ cultures of hybrids of these races (table 4).

### Pathogenicity on Newland

Like Ottawa 770B and Bombay, Newland possesses a single rust-conditioning factor and has been either immune from or highly susceptible to all races of *Melampsora lini* with which it has been tested (*6*). In the crosses of race 22 with races 6 and 24 pathogenicity on Newland was conditioned by a single factor, $A_nA_n$, avirulence being dominant. As shown in tables 2 and 4, fit of the observed to the expected ratio based on this hypothesis was excellent.

Pathogenicity on Newland apparently is inherited independently of that on the other differential varieties tested (table 3). The high $\chi^2$ value for the cultures attacking Newland and avirulent on Williston Brown may be accounted for by the deficiency in the number of $F_2$ segregates to which the latter variety was resistant.

### Pathogenicity on Tammes Pale Blue

Tammes Pale Blue is susceptible to race 22 and practically immune from races 6 and 24 (tables 1 and 4). The hybrids of race 22 × race 24 segregated in a ratio suggesting that a pair of dominant factors, $A_{tpb}A_{tpb}$ in the race 24 parent conditioned avirulence on this variety. The ratio of $F_2$ segregates of the cross of race 6 with race 22 may be explained by assuming that the avirulence of race 6 is conditioned by two duplicate factors, $A_{tpb}A_{tpb}A_{tpb'}A_{tpb'}$.

Studies on the inheritance of factors conditioning rust reaction in

flax (*6*) indicated that the resistance of Tammes Pale Blue was due to a single dominant factor. Unfortunately, neither race 6 nor hybrids of race 6 were used, so that the hypothesis of two race 6 factors avirulent on Tammes Pale Blue is neither substantiated nor refuted. To race 24 and to the race 22 × race 24 hybrids only one rust-conditioning factor was effective. The Tammes Pale Blue factors are not entirely qualitative as are those of Ottawa 770B, Bombay, and Newland. There was a variation in the degree of susceptibility to some $F_2$ cultures. Under very favorable conditions for rust development avirulent cultures sometimes induced the development of minute, usually subepidermal pustules giving a resistant rather than an immune reading. These variations suggested incomplete dominance, but it is possible that minor modifying rust-conditioning factors may be present in Tammes Pale Blue.

The pathogenic factor $A_{tpb}A_{tpb}$ conditioning avirulence of race 24 on Tammes Pale Blue was closely linked or identical with 1 of the 3 race 24 factors for avirulence on Morye. Tammes Pale Blue was highly susceptible to the 3 $F_2$ cultures of race 22 × race 24 to which Morye was susceptible and to 5 of 7 of those to which it was moderately susceptible. Morye was immune from all $F_2$ cultures of race 6 × race 22, and Tammes Pale Blue was susceptible to only 6 of the 90 cultures with which it was tested. Since Akmolinsk, Abyssinian, and Leona were susceptible to each of the 6 cultures attacking Tammes Pale Blue (table 4), it is highly probable that the second factor for avirulence of race 6 on Tammes Pale Blue, $A_{tpb'}A_{tpb'}$, is in the group of linked factors conditioning pathogenicity on these varieties.

### Pathogenicity on Bolley Golden

Reaction to rust in Bolley Golden is conditioned by 2 factors, one of which conditions immunity from and the other resistance to most avirulent races (*6*). The $F_2$ cultures of the cross of race 22 and race 24 segregated for pathogenicity on Bolley Golden in a ratio approximating the 1 virulent to 15 avirulent expected if 2 factors were involved. Bolley Golden was resistant to 27 and immune from 100 of the avirulent cultures. The observed ratio of 6 virulent to 27 moderately avirulent to 100 highly avirulent compared with the theoretical 1:3:12 based on the hypothesis that avirulence of race 24 on Bolley Golden was conditioned by 2 factors, $A_{bg}A_{bg}A_{bg'}A_{bg'}$, one for high avirulence and the other for moderate avirulence, gives a $\chi^2$ value of 0.81, $P$ lying between 0.50 and 0.70, an excellent fit. Apparently race 6 possesses the same factors for avirulence on Bolley Golden as does race 24. The occurrence of 6 virulent to 68 avirulent cultures in hybrid A and of 6 virulent to 85 avirulent in all cultures of hybrids A, B, and C that were tested (table 4) on Bolley Golden satisfactorily approximates the 1:15 ratio expected.

The analysis for independent assortment of factors (table 3) indicates that high avirulence on Bolley Golden and Italia Roma is conditioned by a common or a closely linked factor. Thus, in the cross of race 22 with race 24 (table 1) Italia Roma was susceptible to 2 and had an intermediate reaction (resistant to moderately susceptible) to 4 of the 6 cultures virulent on Bolley Golden. Of the 27 cultures to which Bolley Golden was resistant, Italia Roma was sus-

ceptible to 7, moderately susceptible to 8, and resistant to 10. The immune reading on Italia Roma of the 2 cultures to which Bolley Golden was resistant may have been due to adverse conditions for rust development during these tests.

### Pathogenicity on Italia Roma

Italia Roma possesses two pairs of rust-conditioning factors, one apparently identical with the Bolley Golden factor conditioning immunity and the other independently inherited and conditioning variable degrees of resistance to certain races avirulent on this variety (*6*).

Of the 133 $F_2$ cultures of race 22 × race 24 tested, 9 were virulent on Italia Roma, 27 were intermediate in virulence (resistant to moderately susceptible), and 97 were avirulent (table 1). This approximates the hypothetical 1:3:12 ratio expected if pathogenicity on Italia Roma was conditioned by 2 factors, race 22 carrying the recessives for virulence $a_{b_g}a_{b_g}a_{t_r}a_{t_r}$ and race 24 carrying one dominant factor for avirulence, $A_{b_g}A_{b_g}$, and a second quantitative factor, $A_{t_r}A_{t_r}$, conditioning a variable degree of avirulence.

The occurrence of 18 virulent to 56 avirulent $F_2$ segregates of race 6 × race 22 hybrid A (table 4) very closely approximates the 1:3 ratio expected if a single dominant factor conditioned the avirulence of race 6 on Italia Roma. There were some differences in the degree of susceptibility of Italia Roma to the virulent $F_2$ cultures of race 6 × race 22, indicating that the second rust-conditioning factor in Italia Roma was not wholly ineffective against race 6. The occurrence of no virulent and only 1 moderately virulent $F_2$ culture among the 16 segregates of hybrid B suggests that race 6 was homozygous for one and heterozygous for a second factor, $A_{b_g}A_{b_g}A_{t_r}a_{t_r}$, for avirulence on Italia Roma.

Analysis of the $F_2$ cultures of race 22 × race 24 for independent assortment of pathogenic characters indicates linkage between virulence on Pale Blue Crimped, Kenya, and Italia Roma (table 3). However, no linkage between factors for virulence was apparent in the $F_2$ cultures of race 6 × race 22 (hybrid A). Of the 18 $F_2$ cultures virulent on Italia Roma, 4 were virulent on Pale Blue Crimped and Kenya; this is the approximate number that would be expected on the basis of independent assortment of characters conditioned by single pairs of recessive factors. This indicates that race 24 carried the dominants and race 22 the recessive allelomorphs and that race 6 was heterozygous for the factors conditioning pathogenicity on Italia Roma that were linked with factors for pathogenicity on Pale Blue Crimped and Kenya.

### Pathogenicity on Morye

In Morye, 3 factors condition resistance to North American races of *Melampsora lini* (*6*). As shown in table 1, of the 133 $F_2$ cultures of race 22 × race 24 tested, 3 were virulent, 7 were moderately virulent, and 123 were avirulent on Morye. This ratio approximates the 1:3:60 ratio expected if 3 factors condition pathogenicity on Morye. In race 22 all 3 factors conditioning virulence, $a_{t_P b}a_{t_P b}a_{a_r}a_{a_r}a_{a_r}a_{a_r}$, are recessive. In race 24, $A_{t_P b}A_{t_P b}A_{a_r}A_{a_r}a_{a_r}a_{a_r}$, the 2 dominant factors condition avirulence and a third factor conditions moderate virulence.

Morye was immune from each of the 98 $F_2$ cultures of race 6 × race

22 (table 4). This suggests that race 6 possesses either an additional factor for avirulence or one allelic to the factor that in race 24 conditions moderate virulence on Morye. Since the data are limited and the allelic hypothesis is the simpler in that it does not increase the number of pathogenic factors, it has been assumed that race 6 possesses 3 factors, $A_{1pb}A_{1pb}A_{ar}A_{ar}A_{ar'}A_{ar'}$, each of which conditions a high degree of avirulence on Morye.

The identity or probable linkage of a factor for pathogenicity on Morye with one for pathogenicity on Tammes Pale Blue has been presented on page 352.

## DISCUSSION

The possible consequences of the establishment in the flax-producing region of the northern part of the United States of a race having the pathogenic range of race 22, known to occur in nature in South America only, must be largely speculative. In the northern United States sexual reproduction of *Melampsora lini* precedes the initiation of infection each season. Although it is possible that the imported race might maintain its virulence by natural selfing, it is more probable that hybridization would be the rule, especially if varieties that are susceptible to both North American and South American races continue to be grown. That hybridization would result in at least a temporary loss of virulence is apparent from these studies. The $F_1$ cultures of hybrids of race 22 with race 24, which between them attacked 15 of the 16 differential varieties (table 1), were capable of attacking only 3 because of the almost universal dominance of avirulence. Race 24 possesses at least 5 recessive factors for pathogenicity and race 22 possesses 16 or more. In 1 of 133 $F_2$ cultures of hybrids of these races 10 pairs of these factors were recovered as shown by pathogenicity tests. Consequently, none of the hybrids approached race 22 in virulence. There was no indication of allelomorphic or linkage relation between the factors of race 24 for virulence on Bombay, a variety immune from race 22, and any of the factors for virulence on the 14 differential varieties attacked by race 22. Consequently, it should be possible to obtain a race from this cross attacking all the differential varieties except J. W. S. Vallega (*11*) reported the isolation of a race in Argentina that attacks J. W. S. in addition to the differential varieties attacked by race 22. At the present time there appear to be no theoretical limitations to the accumulation of pathogenic factors in *M. lini* and the evolution of a race capable of attacking all varieties of flax.

The discovery of the specificity of relations between definite factors for rust reaction in the host and those for pathogenicity in the pathogen opens the way for speculation as to the potential number and variability of physiologic races of *Melampsora lini*. From 231 $F_2$ cultures of hybrids of race 6 with race 22 and of race 22 with race 24, 98 different races were identified of which 92 had not been isolated previously. Analysis of the hybrids of race 22 × race 24 for independent assortment of factors conditioning virulence showed that pathogenicity on 8 varieties or groups of varieties was independently inherited and that of 2 varieties in a ninth group, 1, Tammes Pale Blue, was susceptible to all cultures attacking the second, Morye; but

Morye was resistant to some cultures attacking Tammes Pale Blue. Theoretically, this would permit the occurrence of 768 pathogenically distinct races from this 1 cross, based on only 2 categories of host reaction, namely, susceptible and immune or resistant.

Tests in which the hybrid races described in this paper were used as inoculum indicated that the 16 differential varieties possessed at least 19 distinct pairs of rust-conditioning factors (*6*). Studies on the inheritance of pathogenicity in the rust fungi would be facilitated by the development of lines of the host possessing single pairs of rust-conditioning factors. There is no apparent reason why this cannot be done with flax. If this was accomplished, more than a million races could be differentiated by the factors possessed by the varieties used in these studies. Additional rust-conditioning factors are known to occur in other varieties. The writer has found that Light Mauve (C. I. 379–1) possesses 2 pairs and Koto (C. I. 842) 1 pair of rust-conditioning factors not found in the 16 differential varieties. Bison, uniformly susceptible to rust in North America, South America, and Europe, is immune from some races found in Australia (*12*) and consequently possesses at least 1 pair of factors conditioning immunity from Australian races.

The discovery that the virulence of races of *Melampsora lini* is due to the interaction of each of a number of specific factors in the fungus with corresponding specific factors for resistance in the host is significant to those working in flax improvement. The pathogenicity of each race depends upon its content of dominant or recessive factors, and by the hybridization of selected races it should be possible to obtain almost any desired combination of virulent factors. The number of races identifiable on the differential varieties now known is limited only by the diligence and resources of the investigator. Yet the problem is not as complex as indicated by the millions of potential races, since these differ only in their possession of a relatively few factors for pathogenicity. Variations in virulence on the 14 varieties differentiating the races used in these investigations were explained by assuming 18 factors for pathogenicity that occur in 12 independently inherited or linked groups (table 5). It is possible that additional pathogenic factors may be independently inherited. On the other hand, because of the limited number of cultures studied, it is possible that future investigations may show linkage between some of the factors herein reported as being independently inherited.

Races of *Melampsora lini* capable of attacking varieties at present resistant to rust in North America may arise or become established by hybridization, mutation, or introduction. Hybridization probably is coincidental with the development of *M. lini* each season in the principal seed-flax producing region of the United States. However, the discovery of the specificity of the interaction of each factor for pathogenicity in the fungus with each factor for resistance in the host indicates that races arising through hybridization will not attack heretofore resistant varieties. J. W. S., immune from races 6, 22, and 24, was immune from the 231 $F_2$ cultures of hybrids of these races. Bombay, immune from races 6 and 22, was immune from the 98 $F_2$ cultures of this cross. This suggests that different combinations of factors do not interact to increase virulence on or to break down the resistance of an

immune variety possessing distinct rust-conditioning factors. Insufficient cultures were studied to warrant any generalization concerning the role of mutation in the origin of races attacking resistant varieties. What evidence exists is negative. None of the 231 $F_2$ cultures studied attacked J. W. S. and none of the 98 $F_2$ cultures of the cross of race 6 with race 22 attacked Bombay, varieties immune from the parent races. Consequently, it is considered that the greatest danger to our present rust-resistant varieties of flax is the introduction from some other region, particularly South America, of a race that will attack them.

## SUMMARY

The mode of inheritance of pathogenicity in *Melampsora lini* was studied by determining the reaction of 16 differential flax varieties to $F_2$ cultures of hybrids of races 6 and 22 and races 22 and 24. All differential varieties, except Bombay and J. W. S., were susceptible to race 22. Buda was moderately susceptible and Williston Golden, Williston Brown, and Bombay were susceptible to race 24. Only Williston Golden and Williston Brown were susceptible to race 6.

Virulence on all differential varieties except Williston Golden and Williston Brown was inherited as a recessive character. No $F_2$ culture approached parent race 22 in virulence as measured by the number of differential varieties attacked. From the 133 $F_2$ cultures of hybrids of race 22 with race 24, 64 races were identified of which 62 had not been isolated previously. Ninety-eight $F_2$ cultures of hybrids of race 6 with race 22 yielded 39 races of which 36 had not been isolated from field collections.

Segregating ratios and tests for independent assortment of factors indicated that for each pair conditioning rust reaction in the host there is a corresponding pathogenic pair in the rust fungus. The $F_2$ cultures of crosses of race 22 with race 24 segregated for pathogenicity on Pale Blue Crimped, Kenya, Akmolinsk, Abyssinian, Leona, Ottawa 770B, Bombay, Newland, and Tammes Pale Blue, varieties having one pair of factors for resistance to the avirulent parent, in a single-factorial ratio; on Bolley Golden and Italia Roma, varieties having two pairs of factors for resistance to the avirulent parent, in a bifactorial ratio; and on Morye, a variety having three pairs of factors for resistance to the avirulent parent, in a trifactorial ratio. Buda was susceptible in varying degrees to the parent races and all $F_2$ cultures, and J. W. S. was immune from them.

The $\chi^2$ test of $F_2$ cultures of hybrids of race 22 with race 24 for independent assortment showed that factors for virulence on Pale Blue Crimped and Kenya and on Akmolinsk, Abyssinian, and Leona were so closely linked as to be inherited as a unit. Pathogenicity on Williston Golden and Williston Brown, varieties having a common factor for resistance to certain cultures of this cross, also was inherited as a unit. One of the pairs of factors for virulence on Bolley Golden was the same as one of the pairs of factors conditioning virulence on Italia Roma or was closely linked with it. The pair of factors conditioning virulence on Tammes Pale Blue was either the same as one of the three pairs of factors for virulence on Morye or was closely linked with it.

Analysis of the $F_2$ cultures of the hybrids of race 22 with race 24 for independent assortment showed that 768 races having sharp differences in pathogenicity on 1 or more of the differential varieties are theoretically obtainable from this cross.

There was no indication of allelomorphic or linkage relations between the factors for virulence on Bombay, susceptible to race 24, and any of the factors for virulence on the 14 differential varieties attacked by race 22. Consequently, a race attacking all the differential varieties except J. W. S. should be obtainable from this cross.

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# THE NUTRITIVE VALUE OF COTTONSEED MEAL, SOYBEAN MEAL, AND PEANUT MEAL WHEN USED SEPARATELY AND TOGETHER TO SUPPLEMENT THE PROTEIN OF PRAIRIE HAY IN EXPERIMENTS WITH LAMBS [1]

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## INTRODUCTION

In selecting protein supplements for growing, fattening, or wintering sheep, the cost of a unit of crude protein is usually the first consideration. However, a difference in the nutritive value of supplements arising from a difference in protein content is likewise important. Soybean, cottonseed, and peanut meals are readily available supplements that have about the same protein content. The value of soybean meal and cottonseed meal in rations for sheep has been studied in numerous feed lot trials. By means of nitrogen balance studies, Miller and Morrison (*7*) [2] determined the value of the protein in soybean meal for lambs; peanut meal has not been generally used in sheep rations.

Lambs and mature sheep have been employed extensively in digestion and metabolism studies, as have cattle. Comparative studies by Forbes et al. (*4*) have shown that cattle digest crude fiber more efficiently than sheep, but the two species are similar in their ability to digest the protein in natural feeds. The addition of protein supplements to rations low in protein has been found to improve the intake and utilization of feed by lambs. Miller (*6*) has shown that a marked depression of appetite occurs when protein is withheld from lambs.

To determine whether or not differences existed in the nutritive value of the protein in cottonseed meal, soybean meal, and peanut meal these three supplements were fed separately and in combination to growing lambs in nitrogen balance studies. The general methods of investigation correspond closely to those used in a similar study with steers at this station (*2*). The general method and its permissible modifications have been discussed by Mitchell (*9*).

A basal ration of prairie hay of low protein content was fed. The meals were then added as supplements to allow the determination of the apparent digestibility of the nutrients in the complete rations and in the individual feeds. The biological value of the protein of the complete rations was estimated from nitrogen absorbed and retained by the experimental subjects.

## EXPERIMENTAL PROCEDURE

Eight thrifty grade Rambouillet wether lambs were selected from a carload of feeder lambs of Texas origin. The preliminary feeding periods lasted 10 days or more during which time the lambs were confined to small pens. Feces and urine were collected during 10-day periods following the period of preliminary feeding. The lambs were confined to false-bottom metabolism cages similar to the one described

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[2] Italic numbers in parentheses refer to Literature Cited, p. 366.

by Forbes (*3*). The feces were removed daily from the collection screen and dried in an electric oven to approximately air dryness during the next 24 hours. They were then weighed and stored for the remainder of the collection period in airtight glass containers. The urine was collected daily and stored in a glass jug for the remainder of the period. The urine was preserved by the addition of thymol and sulfuric acid.

The lambs were weighed at the start and finish of each collection period. The water was changed twice daily and kept before the lambs at all times. Approximately 10 gm. of salt was mixed into the ration each day. The basal ration consisted of 600 gm. of coarsely ground prairie hay, and the daily allowance of supplement was 73.4 gm. which was fed mixed with the hay. One-half of this mixture was fed in the morning and the other half in the evening. No feed was refused during the experiment.

Locally grown prairie hay that had been cut in late July was fed. Peanut and soybean meals prepared by the hydraulic process were used. The cottonseed meal, obtained from Mississippi, was guaranteed to contain 41 percent protein. The supplements were identical with those fed to steers in a similar study (*2*).

The chemical composition of the feeds is shown in table 1. The feeds and feces were analyzed in accordance with the official methods of the Association of Official Agricultural Chemists (*1*). Nitrogen determinations were made in triplicate on the feed, feces, and urine.

TABLE 1.—*Percentage composition of feeds and rations*[1] *used in digestion and nitrogen-utilization studies with lambs*

| Description of feed or ration | Dry matter | Crude protein (N×6.25) | Ether extract | Crude fiber | N-free extract | Organic matter |
|---|---|---|---|---|---|---|
| Feed: | | | | | | |
| Prairie hay | 90.52 | 4.03 | 2.39 | 32.49 | 46.91 | 85.82 |
| Cottonseed meal | 92.37 | 41.37 | 5.60 | 9.57 | 28.13 | 84.67 |
| Peanut meal | 93.50 | 42.50 | 8.55 | 12.01 | 25.07 | 88.13 |
| Soybean meal | 92.13 | 43.62 | 6.05 | 4.82 | 32.38 | 86.87 |
| Protein mixture[2] | 92.67 | 42.51 | 6.73 | 8.79 | 28.52 | 86.55 |
| Ration: | | | | | | |
| Hay and cottonseed meal | 100.00 | 8.93 | 3.02 | 33.06 | 49.45 | 94.46 |
| Hay and peanut meal | 100.00 | 9.05 | 3.37 | 33.30 | 49.02 | 94.74 |
| Hay and soybean meal | 100.00 | 9.20 | 3.07 | 32.50 | 49.98 | 94.75 |
| Hay and protein mixture[2] | 100.00 | 9.06 | 3.15 | 32.95 | 49.48 | 94.64 |

[1] Composition of the rations is given on an air-dry basis.
[2] The protein mixture consisted of one-third each of cottonseed meal, peanut meal, and soybean meal.

The eight lambs were divided into two groups and while one group was on collection the other four were receiving in the individual pens preliminary rations for the subsequent collection period. All four lambs in a group were fed the same experimental ration during a preliminary and collection period.

The digestibility of the feeds is expressed as the apparent digestion coefficients, while nitrogen retention is expressed as percentage stored and biological value. No direct determinations of metabolic or endogenous nitrogen were made. Miller and Morrison (*7*) have used average values of 0.037 gm. of endogenous nitrogen daily per kilogram of body weight and 0.55 gm. of metabolic nitrogen per 100 gm. of dry-matter intake in a series of metabolism studies. These values are based on an average of several determinations made at the Cornell station. Harris and Mitchell (*5*) reported similar values from feeding a low-nitrogen basal ration. Their values are 0.033 gm. of

endogenous nitrogen per kilogram of body weight and 0.555 gm. of metabolic nitrogen per 100 gm. of dry-matter intake. Swanson and Herman (*12*) reported metabolic nitrogen excretions of 5.3 gm. per kilogram of dry-matter intake for cattle while they estimate daily endogenous nitrogen values in grams from the formula $0.712\ W^{0.42}$.

The data presented have been treated statistically for significance by the analysis of variance method (*11*).

## RESULTS

Table 2 shows the apparent digestibility, calculated total digestible nutrients, and the nutritive ratio of the various rations fed. Prairie hay when fed alone had a coefficient of apparent digestibility of 16.3 percent for protein, 32.4 percent for ether extract, 54.2 percent for crude fiber, 48.2 percent for nitrogen-free extract, and 48.4 percent for dry matter. The coefficients of all supplemented rations were higher than those of the basal prairie hay ration, and these differences were all highly significant except for increase in crude fiber digestibility when peanut meal was added to the prairie hay; in this instance the difference was only 0.5 percent. Although the apparent digestibility of the protein in the basal ration of prairie hay averaged only 16.3 percent, rations supplemented daily with 73.4 gm. of any of the supplements averaged higher than 50 percent.

TABLE 2.—*Average apparent digestibility, digestible nutrient content, and nutritive ratio of rations of prairie hay and of prairie hay supplemented with high-protein meals, as determined with 8 lambs*

| Supplement fed with prairie hay | Apparent digestibility of— | | | | | Total digestible nutrients | Nutritive ratio 1: |
|---|---|---|---|---|---|---|---|
| | Crude protein ($N \times 6.25$) | Ether extract | Crude fiber | N-free extract | Organic matter | | |
| None | 16.3 | 32.4 | 54.2 | 48.2 | 48.4 | 42.6 | 63.9 |
| Cottonseed meal | 52.0 | 40.6 | 59.7 | 55.4 | 56.1 | 49.5 | 10.7 |
| Peanut meal | 58.2 | 58.1 | 54.7 | 53.1 | 54.3 | 49.5 | 9.3 |
| Soybean meal | 59.5 | 47.8 | 61.2 | 56.6 | 58.2 | 51.6 | 9.4 |
| Protein mixture [1] | 56.4 | 49.0 | 59.0 | 55.6 | 56.6 | 50.4 | 9.9 |

[1] The protein mixture consisted of one-third each of cottonseed meal, peanut meal, and soybean meal.

Some differences were found in the digestibility of the various nutrients in the rations when the different supplements were used. The coefficient of apparent digestibility of the protein in the ration supplemented by cottonseed meal was 4.4 percentage units lower than that of the protein mixtures which was next lowest in digestibility. This is a greater difference than the 3.3 percentage units needed for a highly significant difference. The other differences in the apparent digestibility of protein were not significant.

The use of peanut meal as a supplement resulted in apparent digestibility of 54.7 percent of the crude fiber of the ration. This was significantly lower than the value obtained when the protein mixture was used as a supplement and highly significantly lower than when either cottonseed meal or soybean meal was used.

The coefficient of apparent digestibility of the nitrogen-free extract in the ration supplemented with peanut meal was significantly lower than that of the ration supplemented with cottonseed meal or protein mixture, and highly significantly lower than that of the ration supplemented with soybean meal.

Total organic dry matter, like its components, was not as efficiently digested by the lambs when peanut meal was fed as the supplement. The organic dry matter in the ration was more digestible when the protein mixture or soybean meal was the protein supplement. These differences were significant and highly significant, respectively.

The standard error of the difference of nutrient digestibility between rations in the foregoing comparisons was 1.24 for crude protein, 2.29 for ether extract, 1.79 for crude fiber, and 1.06 for nitrogen-free extract and organic matter.

Table 3 gives the apparent digestion coefficients of the supplements as determined by difference. These coefficients were calculated from the data used in obtaining the values presented in table 2. The average coefficients of apparent digestibility for the crude protein in cottonseed meal, peanut meal, soybean meal, and the protein mixture were 80.3, 90.7, 92.1, and 87.5 percent, respectivly. The coefficients for peanut meal, soybean meal, and the protein mixture as found by difference were highly significantly greater than that for cottonseed meal. The coefficient for soybean meal was also highly significantly greater than that for the protein mixture; the standard error of the difference in this comparison was 1.57.

Morrison (*10*) gives the apparent digestion coefficients of the protein in cottonseed meal, peanut meal, and soybean meal as 81, 89, and 85, respectively.

TABLE 3.—*Coefficients of apparent digestibility of protein in supplements as determined by difference in digestion trials with lambs* [1]

| Supplement | Digestibility of protein as determined by difference with lamb No.— | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 59 | 172 | 157 | 118 | 110 | 156 | 139 | 91 | Average |
| Cottonseed meal | 77.3 | 80.2 | 73.8 | 80.8 | 80.6 | 82.6 | 83.9 | 83.4 | 80.3 |
| Peanut meal | 91.0 | 92.9 | 88.5 | 89.8 | 90.0 | 91.2 | 93.6 | 88.7 | 90.7 |
| Soybean meal | 90.7 | 89.7 | 87.7 | 93.8 | 93.9 | 97.1 | 90.8 | 93.1 | 92.1 |
| Protein mixture [2] | 84.3 | 82.5 | 83.4 | 90.6 | 87.6 | 94.2 | 89.5 | 88.1 | 87.5 |

[1] Each supplement was fed in combination with prairie hay in the ratio of 1 part supplement to 8.17 parts of hay.

[2] The protein mixture consisted of one-third each of cotttonseed meal, peanut meal, and soybean meal.

The nitrogen balance data are presented in table 4. Since the lambs were offered the same quantities of feed and it was all consumed there was no difference in nitrogen intake of the lambs receiving a specified ration. Moreover, there were only slight differences in nitrogen intake between the several supplemented rations that each lamb received. Nitrogen storage was decidedly negative for all lambs receiving unsupplemented hay, but was positive when the hay was supplemented. The difference was highly significant. The basal ration of prairie hay provided 3.87 gm. of nitrogen daily, while the nitrogen intake of the supplemented rations ranged from 8.73 gm. to 8.99 gm. daily.

The lambs were more efficient in storing the protein from the rations supplemented with soybean meal, cottonseed meal, or the protein mixture than from the ration in which peanut meal was fed as the protein supplement. These differences were significant. Nitrogen storage on the soybean meal-supplemented ration also showed a significant increase over the storage when cottonseed meal or the protein mixture was used as the supplement. The standard error of the difference in this comparison was 1.64. When expressed as a percentage the total

nitrogen stored during the 10-day periods on the cottonseed meal, peanut meal, soybean meal, and protein mixture rations was 19.9 percent, 15.2 percent, 23.6 percent, and 20.3 percent, respectively.

The average biological value of the protein in the cottonseed meal ration was 79, in the peanut meal ration 69, in the soybean meal ration 76, and in the protein mixture ration 76. The standard error of the difference was 1.64. Consequently, the difference in biological value between the peanut meal ration and the other three was highly significant; the differences between the other supplemented rations was not.

TABLE 4.—*Nitrogen balance data showing utilization by lambs of nitrogen in prairie hay and in rations of prairie-hay and high-protein supplements*

PRAIRIE HAY

| Lamb No. | Body weight | | | Dry-matter intake [1] | Nitrogen intake [1] | Fecal nitrogen | Absorbed nitrogen [2] | Total urinary nitrogen | Feed nitrogen utilized [3] | Total nitrogen stored | Nitrogen | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Initial | Final | Average | | | | | | | | Stored | Biological value |
| | *Kg.* | *Kg.* | *Kg.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Pct.* | |
| 59 | 31.8 | 32.2 | 32.0 | 5,431 | 38.7 | 31.9 | 36.7 | 20.9 | 27.6 | 14.1 | −36.4 | |
| 172 | 31.8 | 32.2 | 32.0 | 5,431 | 38.7 | 33.2 | 35.4 | 16.8 | 30.4 | 11.3 | −29.4 | |
| 157 | 34.0 | 34.0 | 34.0 | 5,431 | 38.7 | 32.5 | 36.1 | 24.7 | 24.0 | 18.5 | −47.4 | |
| 118 | 33.6 | 31.8 | 32.7 | 5,431 | 38.7 | 35.5 | 33.1 | 19.1 | 26.1 | 15.9 | −41.0 | |
| 110 | 24.9 | 25.4 | 25.2 | 5,431 | 38.7 | 32.9 | 35.7 | 13.1 | 31.9 | 7.3 | −18.9 | |
| 156 | 30.8 | 29.0 | 29.9 | 5,431 | 38.7 | 31.8 | 36.8 | 17.4 | 30.5 | 10.5 | −27.1 | |
| 139 | 34.0 | 33.1 | 33.6 | 5,431 | 38.7 | 31.3 | 37.3 | 16.5 | 33.2 | 9.1 | −23.5 | |
| 91 | 34.0 | 32.7 | 33.4 | 5,431 | 38.7 | 30.1 | 38.5 | 12.8 | 38.1 | 4.2 | −10.9 | |
| PRAIRIE HAY AND COTTONSEED MEAL | | | | | | | | | | | | |
| 59 | 30.8 | 31.8 | 31.3 | 6,109 | 87.3 | 43.4 | 77.5 | 29.1 | 60.0 | 14.8 | 17.0 | 77 |
| 172 | 31.8 | 31.8 | 31.8 | 6,109 | 87.3 | 42.0 | 78.9 | 27.5 | 63.2 | 17.8 | 20.4 | 80 |
| 157 | 32.2 | 31.8 | 32.0 | 6,109 | 87.3 | 45.1 | 75.8 | 24.9 | 62.7 | 17.3 | 19.8 | 83 |
| 118 | 31.8 | 30.8 | 31.3 | 6,109 | 87.3 | 41.7 | 79.2 | 27.0 | 63.8 | 18.6 | 21.3 | 81 |
| 110 | 27.7 | 27.7 | 27.7 | 6,109 | 87.3 | 41.8 | 79.1 | 27.6 | 61.7 | 17.9 | 20.5 | 78 |
| 156 | 32.2 | 33.1 | 32.6 | 6,109 | 87.3 | 40.8 | 80.1 | 27.8 | 64.4 | 18.7 | 21.4 | 80 |
| 139 | 32.2 | 31.8 | 32.0 | 6,109 | 87.3 | 40.2 | 80.7 | 31.2 | 61.3 | 16.1 | 18.4 | 76 |
| 91 | 31.8 | 34.5 | 33.2 | 6,109 | 87.3 | 40.4 | 80.5 | 29.3 | 63.5 | 17.6 | 20.2 | 79 |
| Average | | | | | | | | | | | | 79 |
| PRAIRIE HAY AND PEANUT MEAL | | | | | | | | | | | | |
| 59 | 31.8 | 31.8 | 31.8 | 6,117 | 88.6 | 36.9 | 85.3 | 40.3 | 56.8 | 11.4 | 12.4 | 67 |
| 172 | 31.3 | 31.8 | 31.6 | 6,117 | 88.6 | 36.9 | 85.3 | 41.6 | 56.4 | 11.1 | 12.5 | 65 |
| 157 | 31.8 | 31.8 | 31.8 | 6,117 | 88.6 | 38.1 | 84.1 | 38.2 | 57.7 | 12.3 | 13.9 | 69 |
| 118 | 31.3 | 31.3 | 31.3 | 6,117 | 88.6 | 37.5 | 84.7 | 38.4 | 57.9 | 12.7 | 14.3 | 68 |
| 110 | 25.4 | 26.3 | 25.8 | 6,117 | 88.6 | 37.4 | 84.8 | 31.7 | 62.6 | 19.5 | 22.0 | 74 |
| 156 | 29.5 | 29.0 | 29.2 | 6,117 | 88.6 | 36.8 | 85.4 | 38.8 | 57.4 | 13.0 | 14.7 | 67 |
| 139 | 31.8 | 31.8 | 31.8 | 6,117 | 88.6 | 35.6 | 86.6 | 41.9 | 56.5 | 11.1 | 12.5 | 65 |
| 91 | 32.7 | 32.7 | 32.7 | 6,117 | 88.6 | 38.0 | 84.2 | 33.9 | 62.4 | 16.7 | 18.8 | 74 |
| Average | | | | | | | | | | | | 69 |
| PRAIRIE HAY AND SOYBEAN MEAL | | | | | | | | | | | | |
| 59 | 32.2 | 32.2 | 32.2 | 6,107 | 89.9 | 37.2 | 86.3 | 32.3 | 65.9 | 20.4 | 22.7 | 76 |
| 172 | 29.5 | 29.9 | 29.7 | 6,107 | 89.9 | 37.7 | 85.8 | 34.9 | 61.9 | 17.3 | 19.2 | 72 |
| 157 | 32.7 | 32.2 | 32.4 | 6,107 | 89.9 | 38.7 | 84.8 | 29.2 | 67.6 | 22.0 | 24.5 | 80 |
| 118 | 32.2 | 31.8 | 32.0 | 6,107 | 89.9 | 35.6 | 87.9 | 32.6 | 67.1 | 21.7 | 24.1 | 76 |
| 110 | 27.2 | 27.7 | 27.4 | 6,107 | 89.9 | 35.5 | 88.0 | 25.5 | 72.6 | 28.9 | 32.1 | 82 |
| 156 | 33.1 | 33.1 | 33.1 | 6,107 | 89.9 | 33.9 | 89.6 | 35.7 | 66.1 | 20.3 | 22.6 | 74 |
| 139 | 32.7 | 32.7 | 32.7 | 6,107 | 89.9 | 37.1 | 86.4 | 34.2 | 64.3 | 18.6 | 20.7 | 74 |
| 91 | 34.9 | 34.0 | 34.4 | 6,107 | 89.9 | 35.9 | 87.6 | 33.6 | 66.7 | 20.4 | 22.7 | 76 |
| Average | | | | | | | | | | | | 76 |

See footnotes at end of table.

TABLE 4.—*Nitrogen balance data showing utilization by lambs of nitrogen in prairie hay and in rations of prairie-hay and high-protein supplements*—Con.

PRAIRIE HAY AND PROTEIN MIXTURE [4]

| Lamb No. | Body weight | | | Dry-matter intake [1] | Nitrogen intake [1] | Fecal nitrogen | Absorbed nitrogen [2] | Total urinary nitrogen | Fecal nitrogen utilized [3] | Total nitrogen stored | Nitrogen | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Initial | Final | Average | | | | | | | | Stored | Biological value |
| | *Kg.* | *Kg.* | *Kg.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Pct.* | |
| 59 | 31.8 | 31.8 | 31.8 | 6,111 | 88.6 | 40.2 | 82.0 | 32.0 | 61.8 | 16.4 | 18.5 | 75 |
| 172 | 32.2 | 34.0 | 33.1 | 6,111 | 88.6 | 41.1 | 81.1 | 28.6 | 64.7 | 18.9 | 21.3 | 80 |
| 157 | 32.2 | 31.8 | 32.0 | 6,111 | 88.6 | 40.7 | 81.5 | 28.9 | 64.4 | 19.0 | 21.4 | 79 |
| 118 | 31.8 | 31.3 | 31.6 | 6,111 | 88.6 | 37.1 | 85.1 | 29.7 | 67.1 | 21.8 | 24.6 | 79 |
| 110 | 27.2 | 28.6 | 27.9 | 6,111 | 88.6 | 38.6 | 83.6 | 29.4 | 64.5 | 20.6 | 23.3 | 77 |
| 159 | 31.8 | 33.6 | 32.7 | 6,111 | 88.6 | 35.5 | 86.7 | 34.0 | 64.8 | 19.1 | 21.6 | 75 |
| 139 | 31.8 | 32.7 | 32.2 | 6,111 | 88.6 | 37.6 | 84.6 | 36.8 | 59.7 | 14.2 | 16.0 | 71 |
| 91 | 33.6 | 34.5 | 34.1 | 6,111 | 88.6 | 38.3 | 83.9 | 36.2 | 60.3 | 14.1 | 15.9 | 72 |
| Average | | | | | | | | | | | | 76 |

[1] Dry-matter intake and nitrogen values are totals for 10-day period.
[2] Based on an estimated value for the metabolic nitrogen in the feces.
[3] Based on an estimated value for the endogenous nitrogen in the urine.
[4] The protein mixture consisted of one-third each of cottonseed meal, peanut meal, and soybean meal.

## DISCUSSION

The prairie hay used in the experiment contained 4.03 percent crude protein, and when fed to lambs its average apparent digestibility was found to be 16.3 percent. If a correction is made for the metabolic nitrogen in the feces using Miller and Morrison's (*7*) value of 0.55 gm. of nitrogen per 100 gm. of dry matter eaten, a true digestibility coefficient of 93.5 percent is obtained. It thus becomes evident that a large portion of the protein was absorbed from the basal ration of the prairie hay. The average true digestibility coefficients for the protein in the supplemented rations were likewise high. The true digestibility coefficients as calculated for the supplemented rations were 91.0 percent, 96.2 percent, 96.8 percent, and 94.3 percent when the supplements consisted of cottonseed meal, peanut meal, soybean meal, and the protein mixture, respectively. These values indicate that it was not the inability of the lambs to digest the protein from the unsupplemented rations, but rather the large excretion of metabolic nitrogen that brought about the low apparent digestibility of the protein in the basal ration. Mitchell (*8*, *9*), after studying the factors that contribute to the apparent digestibility of protein other than the ability of the animal to absorb the protein from the digestive tract, concluded that a negative correlation exists between the apparent digestibility of a protein and its biological value. Swanson and Herman (*12*) have likewise demonstrated this association.

The apparent digestibility of the ether extract in the rations supplemented with peanut meal was highly significantly greater than that in the other rations, while the ether extract in the soybean meal and protein mixture rations was also highly significantly greater than that in the ration supplemented with cottonseed meal or than the basal ration. The apparent digestibility of the ether extract in the cottonseed meal-supplemented ration was significantly greater than that in the basal ration of prairie hay. However, as pointed out pre-

viously (*2*), the small coefficient of digestibility for ether extract in the hay ration is of doubtful significance since there is little ether extract in hay and many factors affect the accuracy of the determination.

The calculated biological values for the protein of the supplemented rations are in close agreement except those for the peanut meal-supplemented ration. The average value of this ration (69) was lower than the mean of the values of other rations by a highly significant difference. The standard error of the difference between these means was 1.64. In each case the biological value of the rations was higher in experiments with lambs than with steers fed similar hays to which the same supplements had been added. As has previously been shown (*2*), these values determined with steers were higher than those obtained by Swanson and Herman (*12*) with heifers or by Miller and Morrison (*7*) with lambs. These higher calculated values are undoubtedly the result of feeding rations of lower protein content. The dry matter of the supplemented lamb rations contained approximately 9.1 percent crude protein on a dry-matter basis, or 8.2 percent on an air-dry basis. Because of the lower protein intake these biological values should not be compared directly with those of other workers. As previously stated (*2*), the writers find nothing in their data that brings into question the reliability of the biological value concept. However, these values suggest that when the biological values of rations are compared the protein intake should be equalized.

When lambs were fed rations similar to those fed steers (*2*), the lambs digested the protein and ether extract in the ration more efficiently, but the crude fiber less efficiently, than did the steers. Nitrogen-free extract digestion coefficients were strikingly similar for lambs and steers on similar rations.

## SUMMARY

Cottonseed meal, peanut meal, soybean meal, and a mixture of equal parts of the three meals were fed to eight wether lambs, in metabolism cages, and their nutritive values compared. The meals were added to a basal ration of prairie hay that contained 4.03 percent protein and 42.6 percent total digestible nutrients on an air-dry basis. The supplemented rations averaged 9.28 percent protein with a total digestible nutrient content of 50.2 percent and a nutritive ratio of 1:9.8.

The supplemented rations were more digestible than the unsupplemented ration of prairie hay. The coefficients of apparent digestibility for protein in the supplemented rations were 52.0 percent, 58.2 percent, 59.5 percent, and 56.4 percent for the rations supplemented with cottonseed meal, peanut meal, soybean meal, and the protein mixture, respectively. A difference of 2.5 percent could be considered significant and a difference of 3.3 highly significant. When the digestibility of the protein in the meals was calculated by difference, the protein in the cottonseed meal averaged 80.3 percent digestible, in the peanut meal 90.7 percent, in the soybean meal 92.1 percent, and in the mixture 87.5 percent. A difference of 3.2 percent was significant and a difference of 4.3 percent was highly significant. The average digestibility of the protein of the individual meals which composed the mixture was 87.7 percent.

The daily nitrogen intake of the lambs consuming the supplemented rations ranged from 8.73 to 8.99 gm. daily. The coefficients of true

digestibility of the supplemented rations were 91.0 percent, 96.2 percent, 96.8 percent, and 94.3 percent when the supplement was cottonseed meal, peanut meal, soybean meal, and a protein mixture, respectively.

The biological value of the protein of the supplemented rations was 79 for the cottonseed meal ration, 69 for the peanut meal ration, 76 for the soybean meal ration, and 76 for the protein mixture ration. The differences between the value for the peanut meal ration and those for the other supplemented rations were highly significant. These data suggest that as a protein supplement peanut meal was not as effective as the other protein meals with which it was compared. Used in combination with the protein of soybean meal and cottonseed meal, however, no decrease in efficiency was noted. When fed alone the protein mixture was found to have no advantage over cottonseed meal or soybean meal.

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